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Research Article

Integrated biotechnological and molecular strategies for conservation of endangered wild *Brassica* species toward climate-resilient agriculture

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Abstract

Agricultural sustainability is increasingly challenged by climate variability, habitat loss, and erosion of genetic diversity by narrowing the genetic base of cultivated crops. Wild relatives of crop species harbor valuable adaptive traits for climate resilience, yet many remain underexplored and endangered. The present study aimed to develop an integrated conservation framework combining biotechnological propagation, molecular characterization, and ecological reintroduction of endangered wild *Brassica* species. Field surveys were conducted in arid and semi-arid regions of northwestern India to collect ten wild and allied species. In vitro micropropagation protocols were optimized using Murashige and Skoog medium with different hormonal combinations, followed by acclimatization and field reintroduction. Molecular characterization was performed using DNA barcoding (matK, rbcL, and cox2) along with ISSR and SCoT markers to assess genetic identity and diversity. *Brassica tournefortii* exhibited a maximum shoot regeneration rate of 81.2% on medium supplemented with 2.0 mg L^{-1} BAP (6-benzylaminopurine) and 0.5 mg L^{-1} NAA (α-naphthaleneacetic acid), while *B. insularis* showed the highest callus induction (77.9%) at 2.0 mg L^{-1} 2,4-D. Reintroduced plantlets demonstrated a field survival rate of 72%. Molecular analyses revealed 89% interspecific polymorphism and clear ecological clustering among species. This study demonstrates the effectiveness of integrating micropropagation with molecular diagnostics and ecological restoration, highlighting endangered wild *Brassica* species as valuable genetic resources for climate-resilient agriculture.

Keywords: Climate resilience, DNA barcoding, ISSR markers, Micropropagation, *Wild Brassica*

INTRODUCTION

The global food production system is increasingly threatened by climate change, land degradation, and genetic erosion, which together undermine crop productivity and sustainability. The widespread reliance on genetically uniform crop varieties has increased agriculture's exposure to climate extremes and emerging diseases. In this context, wild relatives of domesticated crops harbor genetic traits valuable for breeding climate-resilient varieties, though they remain largely underexploited (Maxted *et al*., 2012; Hajjar and Hodgkin, 2007).

Among the most economically significant plant genera, *Brassica encompasses* oilseed crops such as *Brassica napus (rapeseed) and Brassica juncea (Indian mus-*

tard), as well as vegetables such as Brassica oleracea (cabbage, cauliflower, and broccoli). *Brassica* crops occupy more than 70 million hectares worldwide, ranking second among major oilseed species after soybeans (Zhang *et al*., 2017). Although these crops are of significant agronomic importance, intensive selective breeding has reduced their genetic diversity, making them particularly vulnerable to environmental stresses such as drought, salinity, extreme temperatures, and various diseases (Snowdon *et al*., 2007; Barbetti *et al*., 2014).

Because wild *Brassica* populations evolved in diverse and often harsh environments, they exhibit adaptive features—such as tolerance to drought, salinity, and pests— that are absent from cultivated types (Warwick *et al*., 2006; Rakow, 2004). However, many of these wild taxa are threatened by habitat loss, climate variability, and agricultural expansion, necessitating urgent conservation action (IUCN, 2023). Strategies for their protection must therefore integrate both *ex situ* conservation methods and cutting-edge genomic tools to enable effective characterization and utilization (Pence, 2013; Khoury *et al*., 2014).

Micropropagation is an efficient and reliable ex situ technique for large-scale propagation of rare and threatened plant species. Protocols tailored to wild *Brassica* species have been limited, with most studies focusing on cultivated forms. Efficient tissue culture systems are critical not only for conservation but also for facilitating reintroduction into native habitats, particularly when coupled with genomic data for site- and trait-specific selection (George *et al*., 2008; Debnath, 2020).

Molecular markers—including inter simple sequence repeats (ISSR), start codon targeted (SCoT) polymorphisms, and DNA barcoding based on chloroplast genes such as *matK*, *rbcL*, and *cox2*—have proven to be effective tools for evaluating genetic variability, identifying species, and analyzing population structure within wild germplasm (Kress and Erickson, 2007; Collard and Mackill, 2009). These tools are especially valuable for designing reintroduction strategies that maintain evolutionary integrity and adaptive potential (Heywood, 2017).

The present study addresses these challenges and opportunities through a unified approach that incorporates field surveys, optimized in vitro propagation, and molecular characterization of wild *Brassica* species. Although substantial progress has been made in micropropagation protocols for cultivated *Brassica* species, similar efforts for wild taxa remain underexplored. Furthermore, conservation strategies often lack molecular validation, which is crucial for species authentication, genetic diversity assessment, and population management. Recent studies have emphasised the role of optimised tissue culture protocols in rescuing endangered *Brassica* species through finely tuned hormonal regimes, achieving high regeneration success (Chhikara *et al*., 2025). Additionally, comprehensive molecular characterization using SSR and AFLP markers has proven effective in distinguishing wild species such as *B. insularis* and *B. tournefortii*, revealing eco-geographic divergence and genetic resilience (Chhikara *et al*., 2024). However, these efforts typically focused on either micropropagation or molecular diagnostics in isolation. The present study bridges this gap by integrating in vitro propagation with multi-marker molecular diagnostics, including ISSR, SCoT, and DNA barcoding (matK, rbcL, and cox2), selected for their reproducibility, species resolution capacity, and cost-effectiveness, thereby offering a unified conservation framework for safeguarding and utilizing wild *Brassica* germplasm in the context of climate-resilient agriculture. To the best of our knowledge, this is one of the first attempts to apply a combined biotechnological and molecular approach to the conservation of wild *Brassica* germplasm in the context of climate-resilient agriculture. The primary objectives were: (1) to develop efficient tissue culture protocols for rare *Brassica* species, (2) to assess their genetic diversity using robust molecular markers, and (3) to evaluate survival and phenology of micropropagated plantlets under natural habitat conditions. The ultimate goal was to contribute to climate-resilient agriculture by conserving and harnessing the adaptive potential of wild germplasm. This effort also aligns with India's National Biodiversity Action Plan and international frameworks, such as the Nagoya Protocol, which highlight the importance of conserving and sustainably utilising genetic resources, particularly wild taxa that possess valuable agronomic traits.

MATERIALS AND METHODS

Plant material collection and site survey

Field expeditions were conducted during the 2022–2023 growing seasons across ecologically diverse zones in northwestern India, specifically targeting arid and semi-arid habitats in Rajasthan (Jaisalmer, Barmer, and Bikaner districts) and saline depressions in Haryana and Gujarat. Wild *Brassica* and allied *Brassicaceae* species were identified based on morphological traits and floristic records. GPS-based geotagging was used to record precise collection coordinates for each accession using Garmin eTrex 32x handheld units. *Ten wild species, including Brassica tournefortii, Brassica insularis, Farsetia macrantha, and Draba altaica, were collected and assessed.*

In vitro culture and micropropagation protocols

Explant preparation and sterilization

Seeds and leaf tissues were utilized as explants for culture initiation. Seeds were surface-sterilized using 70% ethanol (30s) and subsequently treated with 0.1%

$HgCl_2$ for approximately 5 min, following the procedure of George *et al*. (2008). Afterward, seeds were thoroughly washed with sterile distilled water to remove any residual disinfectant (George *et al*., 2008). Leaf segments were sterilized with 0.2% sodium hypochlorite for 5 minutes, followed by several rinses with sterile water to ensure complete removal of the sterilizing agent.

Culture media and hormonal combinations

All cultures were maintained on MS basal medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 0.8% agar. Various combinations of cytokinins and auxins were tested to optimize shoot regeneration. The most effective medium for *B. tournefortii* contained 2.0 mg L^{-1} 6-benzylaminopurine (BAP) and 0.5 mg L^{-1} α-naphthaleneacetic acid (NAA), yielding an average shoot regeneration frequency of 81.2%. Culture conditions were 25 ± 2 °C, under a 16 h light/8 h dark regime at a light intensity of ~40 μmol m^{-2} s^{-1} and a relative humidity of approximately 60%. Following shoot elongation, rooting was induced on half-strength MS medium fortified with 1.0 mg L^{-1} indole-3-butyric acid (IBA) (Cardoza and D'Souza, 2002). The regeneration efficiency increased gradually with rising concentrations of BAP and NAA, reaching a peak at treatment T4 (2.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA) with 81.2% shoot regeneration.

Acclimatization and reintroduction

Rooted plantlets were first hardened in polyhouse conditions (28°C, 75% RH) for two weeks using a sterilized soil:sand:vermiculite mix (1:1:1). Thereafter, they were transplanted to arid and semi-arid field sites in Jaisalmer and Hanumangarh for ecological reintroduction. GPS tracking and periodic survival checks were conducted for up to 12 months post-transplant. Data were recorded on survival rate, phenology, flowering, and seed setting.

Genomic DNA extraction and molecular marker analysis

DNA isolation and quality assessment

Genomic DNA was isolated from young leaf tissues of all ten wild accessions using a modified CTAB protocol to effectively eliminate polysaccharides and phenolic compounds (Doyle and Doyle, 1990). The concentration and purity of the extracted DNA were assessed using a NanoDrop spectrophotometer (Thermo Scientific), while DNA integrity was confirmed via electrophoresis on a 0.8% agarose gel.

DNA barcoding

Polymerase chain reaction (PCR) was employed to amplify three chloroplast gene regions—*matK*, *rbcL*, and *cox2*—using gene-specific primers as described by Kress and Erickson (2007). PCR protocols were optimized individually for each primer set. The resulting amplicons were separated on 1.5% agarose gels for visualization and subsequently sequenced in both directions using the Sanger method. Sequence alignment was performed using ClustalW, and species identification was validated by conducting BLAST searches against the NCBI and BOLD reference databases.

ISSR and SCoT marker profiling

A total of 8 ISSR and 6 SCoT primers were selected based on previous studies (Collard and Mackill, 2009). PCR was performed in 25 μL reactions containing 1× Taq buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 10 pmol primer, 1 U Taq DNA polymerase, and 30 ng genomic DNA. The PCR thermal profile consisted of an initial denaturation step at 94°C for 5 minutes, followed by 35 amplification cycles comprising denaturation at 94°C for 30 seconds, primer annealing at 50°C-60°C (depending on the primer set), and extension at 72°C for 1 minute. A final elongation step was carried out at 72°C for 10 minutes. The amplified DNA fragments were resolved on 1.5% agarose gels, stained with ethidium bromide, and visualized using a Bio-Rad gel documentation system. Polymorphism percentages and genetic similarity coefficients were calculated using NTSYSpc, and UPGMA-based clustering was used to construct the dendrogram.

Statistical analysis

Micropropagation data were recorded as mean regeneration frequencies ± standard error (SE) based on three biological replicates (n = 3). One-way analysis of variance (ANOVA) was performed to evaluate differences among treatments, followed by Tukey's HSD post hoc test at $p < 0.05$ using SPSS software (version 24). The detailed ANOVA results are presented in Supplementary Table S1.

RESULTS

Shoot regeneration in *Brassica tournefortii*

To enhance in vitro regeneration, five treatment combinations (T_1–T_5) with varying concentrations of BAP and NAA were evaluated to assess their influence on shoot organogenesis in Brassica tournefortii. The results showed significant variation in regeneration responses depending on hormonal concentration, as summarized in Table 1.

Similarly, the frequency of callus induction in *Brassica insularis* varied significantly across treatments containing different concentrations of 2,4-D (0.5–2.5 mg L^{-1}), as presented in Table 2.

DISCUSSION

The findings of the present study provide compelling

Table 1. Shoot regeneration efficiencies in *Brassica tournefortii* under different concentrations of 6-benzylaminopurine (BAP) and α-naphthaleneacetic acid (NAA)

Treatment	**BAP (mg L^{-1})**	**NAA (mg L□^{1})**	**Shoot regeneration (%)**
T_1	0.5	0.1	42.3
T_2	1.0	0.2	58.6
T_3	1.5	0.3	65.4
T_4	2.0	0.5	81.2
T_5	2.5	0.7	74.7

Table 2. Callus induction frequencies in *Brassica insularis* at varying concentrations of 2,4-D

Treatment	**2,4-D (mg L^{-1})**	**Callus induction (%)**
C_1	0.5	33.5
C_2	1.0	52.0
C_3	1.5	66.8
C_4	2.0	77.9
C_5	2.5	60.4

Where: C_1 = 0.5 mg L^{-1} 2,4-D; C_2= 1.0 mg L^{-1} 2,4-D; C_3 = 1.5 mg L^{-1} 2,4-D; C4 = 2.0 mg L^{-1} 2,4-D; C_5 = 2.5 mg L^{-1} 2,4-D

evidence that the combined application of in vitro propagation, molecular tools, and ecological restoration provides an effective strategy for the conservation of threatened *Brassica* species. In particular, the standardized in vitro protocol for *B. tournefortii*—optimized using a combination of 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA—achieved significantly high shoot regeneration rates (81.2%), indicating a favorable cytokinin–auxin balance for axillary shoot proliferation. This aligns with earlier reports showing that BAP, in synergy with low concentrations of NAA, enhanced shoot regeneration in other *Brassica* species (Cardoza and D'Souza, 2002; Moyo *et al*., 2017).

The decline in regeneration efficiency observed at higher hormonal concentrations (T_5) highlights the physiological sensitivity of explants to hormonal imbalance, which may lead to hyperhydricity or premature senescence—common challenges in tissue culture systems (George *et al*., 2008). Similarly, the callus induction experiment in *B. insularis* revealed a concentration-dependent response to 2,4-D, with 2.0 mg L^{-1} being optimal (77.9%). Beyond this threshold, a decline in callus vigour and frequency was observed, likely attributable to cellular toxicity or hormonal oversaturation, as previously reported in *Arabidopsis* and *Sinapis alba* (Ghasemi *et al*., 2014).

The standardized tissue-culture methods established here enable scalable multiplication and preservation of *wild Brassica* germplasm, which has historically been neglected in mainstream breeding programs. Importantly, the success of field reintroduction trials—where 72% of *B. tournefortii* regenerants survived under desert conditions—demonstrates the ecological viability of re-establishing these species in their native habitats. This ecological viability resonates with findings that species exhibit significant physiological plasticity under elevated CO_2 and thermal regimes, further validating their utility in climate-resilient cropping systems (Kharel *et al*., 2025). Similar rewilding efforts have been proposed for other crop wild relatives as a dual strategy for ecosystem restoration and pre-breeding support (Maxted *et al*., 2012; Redden *et al*., 2015).

Molecular characterization using ISSR and SCoT markers revealed high interspecific polymorphism (89%), with dendrograms clustering species by ecogeographical origin. This aligns with recent genome-wide studies that have employed landscape genomics to map climate-associated genetic variants, enabling targeted conservation in species such as *Rheum palmatum* (Li *et al*., 2025). Such results highlight the underlying genetic structure shaped by local adaptation, and validate the taxonomic identity of collected accessions—critical for both germplasm authentication and trait mining (Collard and Mackill, 2009). DNA barcoding using *matK*, *rbcL*, and *cox2* loci further supported accurate species identification and will enable future traceability in gene banks and reintroduction programs.

From a broader perspective, this study reinforces the strategic value of integrating biotechnological interventions with conservation biology. The endangered *wild Brassica* species under study not only carry genes for abiotic stress tolerance (e.g., drought, salinity) but also possess unique phytochemical and morpho-physiological traits absent in their cultivated counterparts (Warwick *et al*., 2006). Harnessing this diversity through in vitro conservation and molecular profiling can enrich breeding pipelines aimed at developing climate-resilient crops—an urgent necessity given increasing climate volatility and food system instability (Khoury *et al*., 2014; Debnath, 2020).

Moreover, the methodologies and frameworks developed here are broadly transferable to other endangered taxa within the *Brassica*ceae family and beyond. By adopting a conservation-through-use paradigm, this research not only preserves threatened genetic resources but also positions them as active contributors to agricultural innovation and ecological resilience.

Limitations and future directions

While this study offers a robust integrative approach for conserving endangered *Brassica* species, some limitations should be acknowledged. First, the sample size per species was relatively limited due to the scarcity and threatened status of wild populations, which may affect the generalizability of the micropropagation and genetic findings. Second, ecological reintroduction trials were confined to arid and semi-arid zones in northwestern India; future validation across diverse agro-climatic regions is essential to assess wider adaptability. Additionally, the study did not incorporate transcriptomic or whole-genome sequencing, which could further elucidate the molecular basis of stress tolerance. Addressing these limitations in future work will strengthen the application of this conservation-through-use framework at broader geographic and genomic scales.

Conclusion

The findings highlight how integrative biotechnological tools can advance both conservation and sustainable use of endangered *Brassica* species. By successfully standardizing high-efficiency micropropagation protocols and demonstrating ecologically viable reintroduction, we have laid the foundation for scalable conservation models. The strong shoot-regeneration response observed in *B. tournefortii* and the effective callus induction in *B. insularis* highlight the reproducibility and species-specific adaptability of the optimised culture systems. Moreover, the incorporation of molecular diagnostics through DNA barcoding, ISSR, and SCoT markers has not only confirmed species identity but also revealed substantial genetic diversity among the collected accessions. This diversity, often shaped by ecological and geographical pressures, is a valuable asset for future breeding programs aimed at improving crop resilience to biotic and abiotic stresses. The observed polymorphism and ecotype clustering strongly support the premise that these wild taxa can serve as vital gene reservoirs for agronomic traits currently underrepresented in cultivated *Brassica* germplasm. Looking ahead, there is a pressing need to expand such integrated conservation frameworks across additional centers of *Brassica* diversity and other climate-sensitive crop groups. Long-term ecological monitoring of reintroduced populations, combined with genome-wide association studies (GWAS) and transcriptomic profiling, could further elucidate adaptive traits under natural selection. Additionally, developing cryopreservation and synthetic seed technologies may enhance ex situ storage capacities and reduce the costs of large-scale propagation. At a policy and community level, bridging conservation science with farmer-led participatory breeding, seed banks, and climate-resilient agricultural strategies will be essential. The conservation-through-use approach adopted here not only protects biodiversity but actively engages it in strengthening food security under uncertain climatic futures. By preserving these endangered species today, genetic tools can be secured for sustainable agriculture.

Supplementary information

The author(s) is responsible for the content or functionality of any supplementary information. Any queries regarding the same should be directed to the corresponding author. The supplementary information is available for download from the article's webpage and will not be included in the print copy.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Barbetti, M. J., Banga, S. S. & Salisbury, P. A. (2014). Challenges for crop production and management from pathogen biodiversity and diseases under current and future climate scenarios—Case study with oilseed *Brassicas*. *Field Crops Research*, 166, 21–32. https://doi.org/10.1016/j.fcr.2014.05.002
2. Cardoza, V. 7D'Souza, L. (2002). Micropropagation of *Brassica* species and their hybrids. *Plant Cell, Tissue and Organ Culture*, 69(2), 177–185. https://doi.org/10.1023/A:1016036308940
3. Chhikara, A., Kumar, V., Sharma, V.&Sharma, S. (2024). Morphological and molecular characterization of *Brassica insularis* and *Brassica tournefortii*. *African Journal of Biomedical Research*, 27(3S), 4201–4208. https://doi.org/10.53555/AJBR.v27i3S.3137
4. Chhikara, A., Kumar, V., Sharma, V.&Singh, D. (2025). Standardization and optimization of in vitro micropropagation techniques for endangered *Brassica* species. *International Journal of Environmental Sciences*, 14(1), 22–28.
5. Collard, B. C. Y. & Mackill, D. J. (2009). Start codon targeted (SCoT) polymorphism: A simple, novel DNA mark-

er technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reporter*, 27(1), 86–93. https://doi.org/10.1007/s11105-008-0060-5

6. Debnath, S. C. (2020). Micropropagation and conservation of crop wild relatives. *Plants*, 9(4), 469. https://doi.org/10.3390/plants9040469
7. Doyle, J. J. & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12, 13–15.
8. George, E. F., Hall, M. A.&De Klerk, G. J. (2008). Plant propagation by tissue culture (3rd ed.). Springer.
9. [9] Ghasemi, R., Shariatpanahi, M. E.&Farsi, M. (2014). Callus induction and plant regeneration in *Sinapis alba*. *Biologia Plantarum*, 58(4), 709–713. https://doi.org/10.1007/s10535-014-0452-2
10. Hajjar, R. & Hodgkin, T. (2007). The use of wild relatives in crop improvement: A survey of developments over the last 20 years. *Euphytica*, 156, 1–13. https://doi.org/10.1007/s10681-007-9363-0
11. Heywood, V. H. (2017). Plant conservation in the Anthropocene – challenges and future prospects. *Plant Diversity*, 39(6), 314–330. https://doi.org/10.1016/j.pld.2017.10.004
12. IUCN (2023). The IUCN Red List of Threatened Species. Version 2023-1. https://www.iucnredlist.org (Accessed on 21 June 2025).
13. Kharel, T. P., Reddy, K. R., Sehgal, A., Kodadinne, N., Bheemanahalli, R., Bhandari, A. B., Reddy, K. N.&Gao, W. (2025). Impact of climate change stressors—temperature, CO_2, and UV-B—on early growth and development of different cover crop species. Agrosystems, *Geosciences & Environment*, 8(1), e70037. https://doi.org/10.1002/agg2.70037
14. Khoury, C. K., Amariles, D. & Soto, J. S., *et al*. (2014). Increasing homogeneity in global food supplies and the implications for food security. *PNAS*, 111(11), 4001–4006. https://doi.org/10.1073/pnas.1313490111
15. [15] Kress, W. J. & Erickson, D. L. (2007). A two-locus global DNA barcode for land plants. *PLoS ONE*, 2(6), e508. https://doi.org/10.1371/journal.pone.0000508
16. [16] Li, F., Wang, C., Zhou, L., Wang, Y., Wang, J., Wang, Z., Zhou, T.&Wang, X. (2025). Harnessing landscape genomics to evaluate genomic vulnerability and future climate resilience in an East Asia perennial. bioRxiv. https://doi.org/10.1101/2025.01.19.633819
17. Maxted, N., Kell, S. P., Ford-Lloyd, B. V., Dulloo, M. E.&Toledo, Á. (2012). Towards the systematic conservation of crop wild relatives. *Crop Science*, 52(2), 774–785. https://doi.org/10.2135/cropsci2011.08.0415
18. Moyo, M., Finnie, J. F.&Van Staden, J. (2017). The effects of plant growth regulators on regeneration in *Brassica napus*. *Plant Cell, Tissue and Organ Culture*, 130, 667–678. https://doi.org/10.1007/s11240-017-1252-6
19. Murashige, T. &Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
20. Pence, V. C. (2013). In vitro methods and the challenge of exceptional species for target 8 of the global strategy for plant conservation. *Annals of the Missouri Botanical Garden*, 99(2), 214–220 https://doi.org/10.3417/2011113
21. Rakow, G. (2004). Species origin and economic importance of *Brassica*. In E. C. Pua & C. J. Douglas (Eds.), *Biotechnology in Agriculture and Forestry* (54, 3–11). Springer.
22. Redden, R., *et al*. (2015). Genetic resources and conservation of crop wild relatives for climate adaptation. *In Genomics of Plant Genetic Resources,* (411–437). Springer.
23. [Snowdon, R. J., Lühs, W.&Friedt, W. (2007). Oilseed rape. In C. Kole (Ed.), *Genome Mapping and Molecular Breeding in Plants*, (2, 55–114). Springer.
24. Warwick, S. I., Francis, A.& Susko, D. J. (2006). The biology of Canadian weeds. 132. *Brassica* napus *L.* and *Brassica rapa L. Canadian Journal of Plant Science*, 86 (4), 981–1001. https://doi.org/10.4141/P04-067
25. Zhang, H., Zhang, D., Wang, M., Sun, J., Qi, Y., Li, J., Wei, X.&Han, L. (2017). Genetic diversity and population structure of *Brassica* napus revealed by SSR markers. PLoS ONE, 12(3), e0174707. https://doi.org/10.1371/journal.pone.0174707